IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Keiji KAMIYAMA et al. : Docket No.: 2004_1927A
Serial No. 10/517,633 : Group Art Unit: 1625
Filed December 13, 2004 : Examiner: Patricia L. MORRIS
For: PRODRUGS OF IMIDAZOLE DERIVATIVES, FOR USE AS PROTON PUMP INHIBITORS IN THE TREATMENT OF E.G. PEPTIC ULCERS

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner for Patents,
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Hiroshi Banno, the undersigned, a citizen of Japan residing at 1-21, Hagihara 2-chome, Kawanishi-shi, Hyogo 666-0004, JAPAN do hereby declare:

That I am an employee of the Assignee of the above-identified application,

That I graduated from Nagoya University with degree of Master of Engineering in March 1991,

That I have been employed by Takeda Chemical Industries, Ltd. (now, Takeda Pharmaceutical Company Limited), Osaka, Japan, since April, 1991, and have been engaged in pharmaceutical research of said company,

That I am a member of the pharmaceutical Society of Japan, and published with other research workers, a number of reports on scientific studies, among others, including Miki, T.; Kori, M.; Mabuchi, H.; Banno, H.; Tozawa, R.; Nakamura, M.; Itokawa, S.; Sugiyama, Y.; Yukimasa, H. *Bioorg. Med. Chem.*, 2002, *10*, 401.

That I am one of the inventors of the above-identified patent application,

That the following experiments were carried out by myself and under my direction:

EXPERIMENTS

1. Object of the Experiments

Cited Reference, Rainer et al. (U.S. Patent No 4,686,230, hereinafter '230) disclose substituted carbamoyl groups such as dimethylcarbamoyl group as substituents for R^5 of the compound (I).

Using N,N-dimethyl-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfinyl]-1H-benzimidazol-1-carboxamide, which has a dimethylcarbamoyl group as disclosed in '230 (hereinafter abbreviated as Compound A) and various example compounds according to the present invention as samples, hydrolysis tests using hepatic S9 and intestinal S9 were carried out to confirm whether or not these compounds act as a prodrug.

2. Synthesis of Compound A

A mixture of 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfinyl]-1H-benzimidazol (0.739 g), dimethylcarbamoyl chloride (0.276 mL), triethylamine (0.418 mL) and tetrahydrofuran (20 mL) was stirred at 60°C overnight. To the reaction solution was added a saturated aqueous solution of sodium bicarbonate (100 mL), and the mixture was extracted with ethyl acetate (100 mL). The ethyl acetate layer was washed with saturated brine (50 mL) and dried over anhydrous magnesium sulfate. The solution was concentrated under reduced pressure. The residue was

purified by silica gel column chromatography (eluted with ethyl acetate:tetrahydrofuran = 1:0 then 1:1), which was then crystallized from diethyl ether-diisopropyl ether to give Compound A (0.767 g) as a colorless solid.
^{1}H-NMR ($CDCl_3$): 2.24 (3H, s), 3.11 (6H, s), 4.38 (2H, q, J=7.9 Hz), 4.91 (1H, d, J=13.4 Hz), 5.04 (1H, d, J=13.4 Hz), 6.65 (1H, d, J=5.5 Hz), 7.34-7.51 (3H, m), 7.81-7.90 (1H, m), 8.36 (1H, d, J=5.5 Hz).

3. Hydrolysis test method

3.1. Samples

Hepatic S9: purchased from Nosan Corporation
(manufactured by XenoTech)
Rat (Cat. No. R4000.S9, Lot No. 0010147)
Dog (Cat. No. D1000.S9, Lot No. 0010189)
Human (Cat. No. H0610.S9, Lot No. 063099A)

Intestinal S9: purchased from Nosan Corporation
(manufactured by XenoTech)
Rat (Cat. No. R1000.IS9, Lot No. 0110077)
Dog (Cat. No. D1000.IS9, Lot No. 0110079)
Human (Cat. No. H0610.IS9, Lot No. 0110085)

3.2. Composition of reaction solution (final concentration)

Test Compound	10 μmol/L
Hepatic and intestinal S9	each 1.0 mg protein/mL
Phosphate buffer (pH 7.4)	0.05 mol/L

3.3. Reaction

The sample was preincubated for 5 minutes, and to

this sample was added a test compound to initiate a reaction. The reaction was carried out at 37°C for 15 minutes. A part of the reaction solution was taken immediately after the initiation of the reaction, which was used as a control solution. After the reaction was completed, an equivalent amount of acetonitrile was added to the reaction solution and the mixture was stirred to quench the reaction. The solution was then centrifuged at 15000 rpm for 10 minutes to obtain a supernatant, and said supernatant was used as a sample solution. The sample solution and control solution (50 μL each) were injected to an HPLC column to measure the peak areas, and the percentages of the remaining (unhydrolyzed) prodrug and lansoprazole (parent compound, optical purity was not measured) were calculated using the thus-obtained peak areas of the sample solution and control solution.

During this calculation, the molar absorption coefficient of lansoprazole was assumed and found to be the same as that of the unhydrolyzed compound.

3.4. HPLC condition

Column: CAPCELL PAK C18 MG
(particle size 3 μm, 4.6 mm i.d.× 75 mm)
Mobile phase A: 10 mmol/L CH_3COONH_4/CH_3CN (9:1)
Mobile phase B: 10 mmol/L CH_3COONH_4/CH_3CN (1:9)
0-7 min 37.5% → 100% (B)
7-10 min 100% (B)
10-15 min 37.5% (B)
Column temp.: 40°C
Flow rate: 1 ml/min
Detection: UV 280 nm

4. Results of hydrolysis tests using hepatic S9 and intestinal S9 for the compounds of the present invention and Compound A

The results are shown in the following Tables 1 to 3.

Table 1

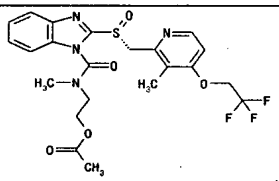
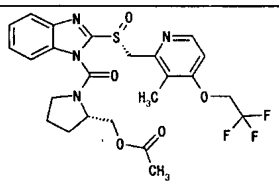
Compound		hepatic S9			intestinal S9		
		rat	dog	human	rat	dog	human
 Ex.1	Lansoprazole release (%)	127	98	119	122	31	103
	Prodrug remaining (%)	2	1	0	1	65	11
 Ex.30	Lansoprazole release (%)	109	116	118	123	35	113
	Prodrug remaining (%)	1	0	0	0	58	1

Table 2

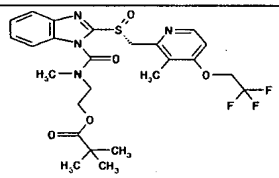
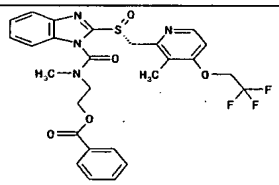
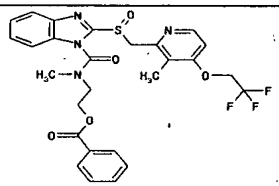
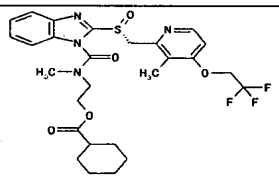
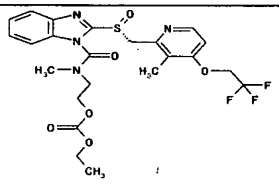
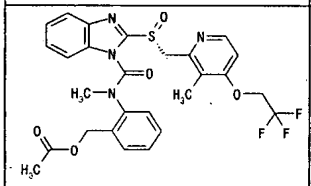
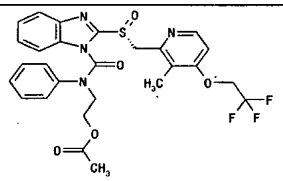
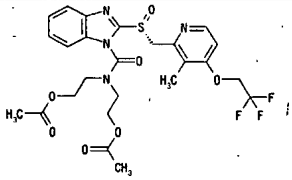
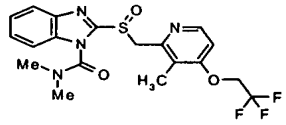
Compound		hepatic S9			intestinal S9		
		rat	dog	human	rat	dog	human
 Ex.2	Lansoprazole release (%)	41	80	116	126	2	113
	Prodrug remaining (%)	62	37	0	0	87	0
 Ex.4	Lansoprazole release (%)	91	57	102	116	2	102
	Prodrug remaining (%)	0	32	0	0	99	0
 Ex.5	Lansoprazole release (%)	96	80	107	121	2	98
	Prodrug remaining (%)	13	21	0	0	98	0
 Ex.3	Lansoprazole release (%)	103	101	117	126	13	111
	Prodrug remaining (%)	0	0	0	0	69	0
 Ex.14&Ex.48	Lansoprazole release (%)	97	74	117	128	2	114
	Prodrug remaining (%)	14	33	0	0	89	0
 Ex.28	Lansoprazole release (%)	104	94	113	126	44	105
	Prodrug remaining (%)	0	0	0	0	65	0

Table 3

Compound		hepatic S9			intestinal S9		
		rat	dog	human	rat	dog	human
 Ex.25	Lansoprazole release (%)	104	117	112	127	83	103
	Prodrug remaining (%)	1	1	1	0	9	0
 Ex.29	Lansoprazole release (%)	112	117	117	130	33	111
	Prodrug remaining (%)	3	2	2	2	65	5
 Compound A	Lansoprazole release (%)	0.4	0	0.5	0	0	0
	Prodrug remaining (%)	99.9	103.0	99.8	98.8	101.1	102.2

5. Conclusion

It has been evidenced that the prodrug disappeared and the parent compound (lansoprazole) was generated in the example compounds of the present invention, whereas in the case of Compound A, the prodrug mostly remained and the parent compound was hardly generated.

It is declared by the undersigned that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Osaka, Japan, this 4th day of October, 2007

/Hiroshi Banno/

Hiroshi Banno